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Strigula sinoaustralis sp. nov. and three *Strigula* spp. new to China

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Abstract—A new foliicolous lichen is described from South China. *Strigula sinoaustralis* is most similar to *S. concreta* in ascospore dimensions but differs by its white-punctate thallus with entire margins and its longer asci. An analysis of its relationships based on molecular phylogeny is given. *Strigula antillarum*, *S. laureriformis*, and *S. prasina* are reported as new to China.

Key words—new species, taxonomy, phylogenetic analysis, lichenized *Ascomycota*

Introduction

Strigula Fr. (*Strigulaceae*) is a genus of lichenized fungi containing about 70 species (Hyde et al. 2013). Seventeen *Strigula* species (twelve foliicolous; five corticolous or saxicolous) have been recorded from China (Santesson 1952, Wei & Jiang 1991, Aptroot & Seaward 1999, Aptroot & Sipman 2001, Aptroot 2003, Aptroot et al. 2003, Lücking 2008). During our ongoing studies of lichenized fungi in China, we found material representing an undescribed *Strigula* species and three *Strigula* species new to China, which are reported here.

Materials & methods

Lichen collections

Most *Strigula* specimens in this study were collected from Guangxi, Hainan, Yunnan, and Guangdong provinces and are preserved in the Fungarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS-L); one additional collection from Xizang is preserved in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN).

Phenotypic analysis

A Leica M125 dissecting microscope was used for the morphological studies and a Zeiss Axioscope2 compound microscope with a Zeiss Axio Imager A2 for the anatomical studies. Sections were studied in tap water and photographed with an AxioCam MRc5 camera. Thin-layer chromatography (TLC) (Culberson & Kristinsson 1970, Culberson 1972, Orange et al. 2001) was used to detect lichen substances.

Table 1. Specimens of *Strigula* spp. and *Falciformispora* outgroup used in the phylogenetic analysis

Species	Voucher Specimen	GenBank No.
S. antillarum	HMAS-L 0137209	KX216696*
	HMAS-L 0137208	KX216697*
	HMAS-L 0137211	KX216702*
S. prasina	HMAS-L 0137213	KX216700*
	HMAS-L 0137212	KX216701*
S. smaragdula	KoLRI016344	KF553663
	LFF016344	KF553664
S. sinoaustralis	HMAS-L0137203 (T)	KX216699*
	HMAS-L0137204	KX216698*
F. senegalensis	IP614.60	KP132365
F. tompkinsii	IP559.60	KP132366

* = sequences newly generated for this study by the authors

DNA extraction, amplification, and sequencing

Seven fresh specimens representing three different *Strigula* species reported from China were chosen for DNA extraction (Table 1). The extraction procedure followed a modified CTAB method (Rogers & Bendich 1988). The nuclear ribosomal RNA gene region, including the internal transcribed spacer (ITS), was amplified through PCR using the primer pair ITS5 and ITS4 (White et al. 1990). Reactions were carried out in 25 µl reaction volume including 1 µl DNA, 1 µl each primer (10 µM), 2 µl dNTP (2.5 mM each), 2.5 µl amplification buffer (10×, 25 mM $MgCl_2$ contained), 0.5 µl Taq polymerase (rTaq DNA Polymerase, 5 U/µl), 17 µl ddH_2O. Cycling parameters were set to an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min. The new sequences generated for this study were deposited in GenBank (Table 1).

Phylogenetic analysis

Eleven sequences including seven sequences were newly generated for this study (Table 1) were aligned with the program MAFFT (Katoh 2002); two other accessions of *Strigula smaragdula* Fr. were retrieved from GenBank (Jayalal et al. 2013). Due to lack of ITS sequences of other genera in *Strigulales*, two species, *Falciformispora tompkinsii* (El-Ani) S.A. Ahmed et al. and *Falciformispora senegalensis* (Segretain et al.) S.A. Ahmed et al., belonging to *Pleosporales* (the family most closely related to *Strigulales*), were chosen

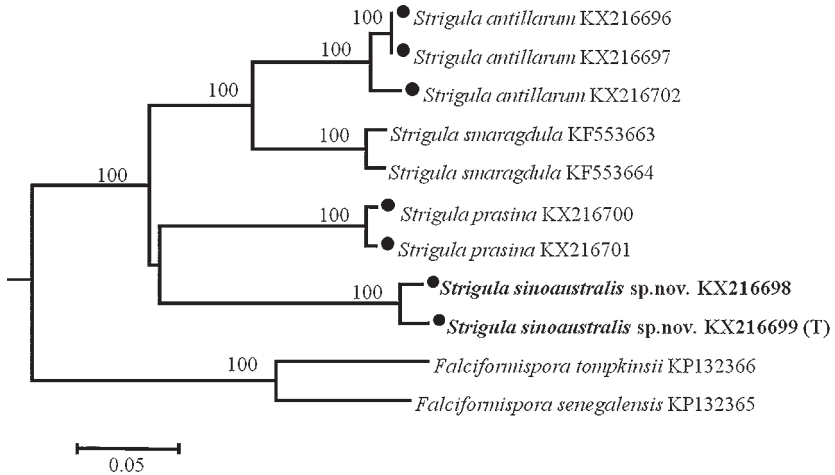

Fig. 1. The Neighbor-Joining tree based on nrDNA ITS sequences. Samples marked with '●' were sequenced by the authors; others were downloaded from GenBank. Genetic distance scale = 0.05 changes per site. Numbers above each node represents bootstrap support values >50%.

as outgroup. The alignment was subjected to a Neighbor-Joining analysis involving 1000 replicates with MEGA (Tamura et al. 2011), using the p-distance model.

Results

The best-scoring Neighbor-Joining tree, based on the eleven ITS sequences (481 bp), is shown in Fig. 1. Within the well-supported (bootstrap = 100%) monophyletic four-species clade of *Strigula*, the new species *S. sinoaustralis* and the Chinese material of *S. antillarum* and *S. prasina* each formed a separate well-supported branch (bootstraps = 100%).

Taxonomy

Strigula sinoaustralis S.H. Jiang, X.L. Wei & J.C. Wei, sp. nov. Pl. 1

Fungal Names FN570267

Differs from *Strigula concreta* by its white-punctate thallus with entire margins and its longer asci.

Type: China. Guangxi Province, Shangsi County, Shiwan mountain forest park, along the way to Yingkesong and Jiulongsong, 21°54′16″N 107°54′09″E, alt. 271 m, on living leaves, 26/V/2015, Xin-Li Wei & Jiao-Hong Wang GX20150342 (**Holotype,** HMAS-L 0137203; GenBank KX216699).

Etymology: The epithet *sinoaustralis* refers to South China.

Thallus subcuticular, dispersed into rounded patches with entire margins, without forming distinct lobes, 2–11 mm across and 25–35 µm thick; surface smooth, grayish green, often minutely white-punctate. Photobiont a *Cephaleuros* sp., composed of angular-rounded cells, 8–14 × 4–6 µm.

Perithecia exposed, almost conical, basal part somewhat broadly spreading, 0.25–0.4 mm diam. and 100–180 µm tall, black. Excipulum prosoplectenchymatous, 10–18 µm thick, brown; involucrellum 22–40 µm thick, carbonaceous, black; paraphyses unbranched. Asci cylindrical or almost thread like, numerous and dense, 72.5–92.5 × 4–5 µm. Ascospores 8 per ascus, uniseriate, ellipsoid, 1-septate, usually with 1–2 oil droplets per cell when fresh, distinctly constricted at the septum and sometimes broken into parts (fragmented) outside the asci, 8–12 × 2.5–3 µm (4–6 µm long for each fragment), 3–4 times as long as broad.

Pycnidia usually rather few, wart-shaped, 0.05–0.15 mm diam.; two types present: one type 0.1–0.15 mm diam. producing macroconidia that are bacillar, 0–1-septate, 4–6 × 1.8–2.3 µm, with a mucilaginous appendage at one or both ends, 5–10 µm long; the second type 0.05–0.1 mm diam., rather few, and often empty. Microconidia not seen.

Chemistry. No substances detected by TLC.

Ecology & substrate. Found in humid, partly exposed habitats on living leaves of bamboo.

> Additional specimens examined: **CHINA**. **Guangdong Province**, Shixing County, Chebaling national nature reserve, along the way to Xianrendong Village, 24°44′7″N 114°12′31″E, alt. 486 m, on living leaves, 15/V/2015, X.L. Wei & J.H. Wang GD2015042-8 (HMAS-L 0137206). **Guangxi Province**, Shangsi County, Shiwan mountain forest park, along the Shitou River, 21°54′3″N 107°54′26″E, alt. 342 m, on living leaves, 25/V/2015, X.L. Wei & J.H. Wang GX20150265 (HMAS-L 0137204; GenBank KX216698), GX20150387 (HMAS-L 0137205).

Remarks: Two other *Strigula* species with carbonized perithecia develop similarly white-punctate thalli: *S. lacericola* P.M. McCarthy, which can be distinguished by its shallowly lobate thallus margins and very narrow (5–7 times longer than broad) ascospores that do not separate either within or outside the asci (McCarthy 2009) and *S. albomaculata* Sérus., which has longer asci (120–140 × 5–8 µm) and larger ascospores (18–22 × 5–6 µm) and macroconidia (8–8.5 × 1.5–2 µm) (Aptroot et al. 1997).

Anatomically, the new species is most closely related to *S. concreta* (Fée) R. Sant. Both share the same perithecial anatomy and small, uniseriate ascospores that fragment outside the asci, but they differ in thallus morphology and ascus dimensions: *S. concreta* lacks white dots on the thallus and has lobulate thallus margins and shorter asci (30–70 × 4–6 µm; Santesson 1952).

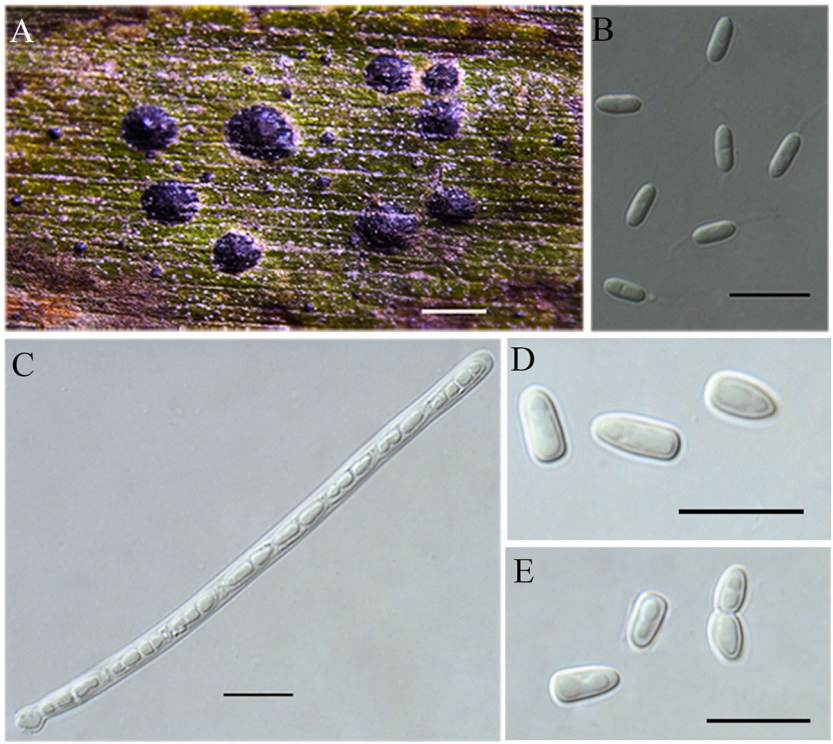

Plate 1. *Strigula sinoaustralis*. A. Thallus with perithecia (holotype, HMAS-L 0137203); B. Macroconidia, with appendage at one or both ends (HMAS-L 0137204); C. Ascus, with eight uniseriate ascospores (holotype, HMAS-L 0137203); D, E. Ascospores, with distinct constriction at septum and breaking into parts outside the asci (holotype, HMAS-L 0137203). Scale bars: A = 300 μm; B–E = 10 μm.

The new species also resembles *S. nitidula* Mont., which is distinguished by a very thin (8–15 μm) marginally effuse bright metallic green thallus usually bordered by a narrow black line (Lücking 2008).

Strigula microspora Lücking can be separated from *S. sinoaustralis* by its thallus with a white central part surrounded by crenulate or shortly lobulate margins, ascospores that do not fragment outside the asci, and larger macroconidia (8–10 × 2–2.5 μm; Lücking 1991, 2008).

Strigula sinoaustralis cannot be confused with any species of the *S. smaragdula* group, which is characterized by broader asci and larger biseriate ascospores (Santesson 1952, Lücking 2008). Besides the morphological differences, the new species is also supported by the phylogenetic analysis (see p. 797).

Strigula antillarum (Fée) Müll. Arg., Bot. Jahrb. Syst. 6: 379 (1885). Pl. 2A–E

Thallus often medium green, 0.5–7.5 mm diam.

Perithecia exposed, immersed at the base, not sharply delimited from the thallus, not easily found in the specimens bearing pycnidia. Asci 55–87.5 × 8–12 µm. Ascospores 8 per ascus, biseriate, fusiform, with acute ends, 17.5–25 × 4–7.5 µm.

Pycnidia producing macroconidia aggregated. Macroconidia bacillar, 1-septate, 12.5–20 × 3–5 µm.

Chemistry. No substances detected by TLC.

Specimens examined: **CHINA**. **Guangxi Province**, Nanning, Long'an County, Longhu mountain natural reserve, 22°57′42″N 107°37′40″E, alt. 147 m, on living leaves, 1/XII/2015, S.H. Jiang GX201511110 (HMAS-L 0137209; GenBank KX216696), GX201511112 (HMAS-L 0137208; GenBank KX216697), GX201511114 (HMAS-L 0137207). **Hainan Province**, Dongfang city, Nanlang village E'xian Ling, 19°00′18″N 109°04′09″E, alt. 160 m, on living leaves, 13/XII/2014, J.H. Wang & R.D. Liu HN2014376 (HMAS-L 0130571), HN2014354 (HMAS-L 0130622); 19°00′07″N 109°04′20″E, alt. 126 m, J.H. Wang & R.D. Liu HN2014393 (HMAS-L 0130573). **Yunnan Province**, Xishuangbanna, Mengla County, tropical botanical garden of Chinese Academy of sciences, East area, 21°55′39″N 101°15′52″E, alt. 560 m, on living leaves, 18/XI/2015, X.L. Wei & S.H. Jiang XTBG2015051 (HMAS-L 0137211; GenBank KX216702); Lvshilin, 21°54′35″N 101°16′52″E, alt. 626 m, 18/XI/2015, X.L. Wei & S.H. Jiang XTBG2015119 (HMAS-L 0137210).

Remarks: *Strigula antillarum* is characterized by its aggregated and confluent pycnidia in the center of the thallus. This species is anatomically similar to *S. smaragdula*, which is distinguished by its thallus-covered perithecia and dispersed pycnidia (Lücking 2008).

Strigula laureriformis Aptroot & Lücking, Biblioth. Lichenol. 97: 131 (2008). Pl. 2F,G

Thallus growing on bark, bright green to white green.

Pycnidia immersed-erumpent, wart-shaped, covered by thallus, with ostiole exposed outside. Conidia ellipsoid, 1-septate, hyaline, 7.5–10 × 2.5–4 µm, sometimes with appendage 15–25 µm long.

Perithecia not seen.

Chemistry. No substances detected by TLC.

Specimens examined: **CHINA**. **Xizang Province**, Motuo County, alt. 1000 m, on living leaves, 1982, Y.G. Su 2532 (KUN 7537).

Remarks: *Strigula laureriformis* (very typical of the genus; Aptroot et al. 2008) does not produce perithecia and is easily recognized by its corticolous habit, bright white green thallus, and simple 1-septate conidia.

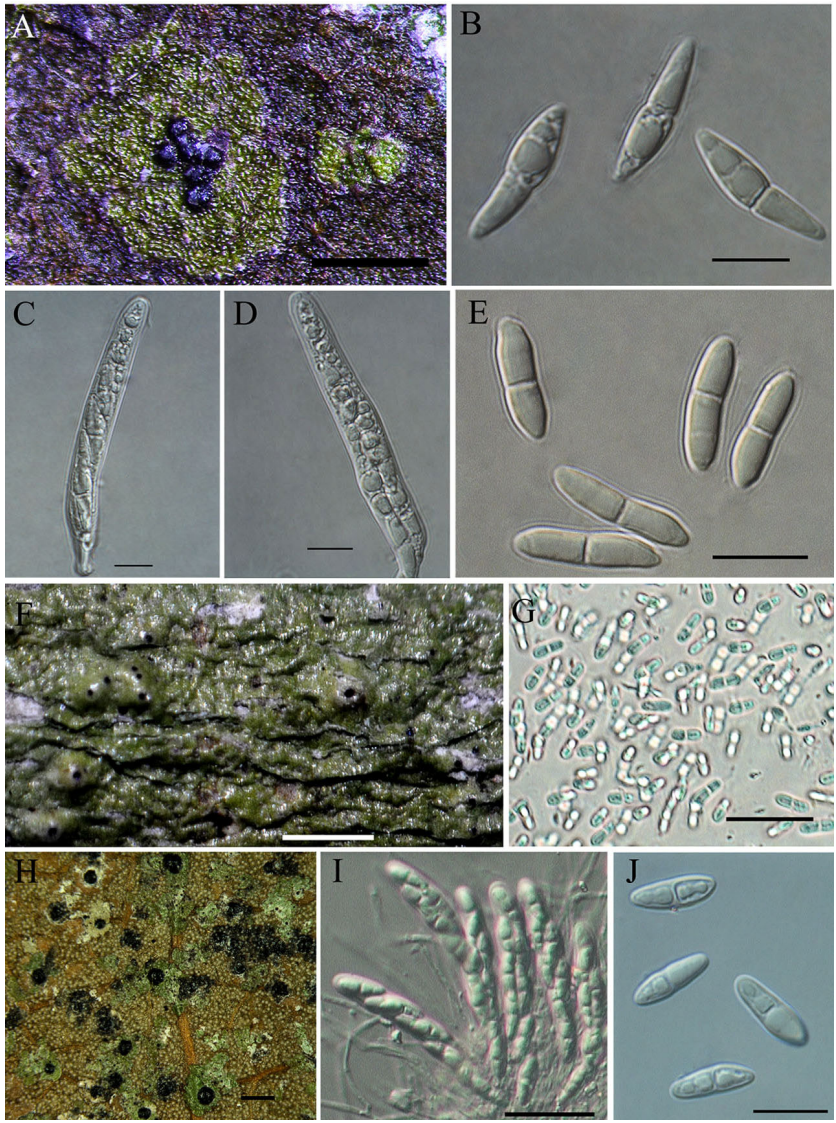

Plate 2. ***Strigula antillarum***. A. Thallus with pycnidia (HMAS-L 0137209); B. Ascospores, fusiform, with acute ends, not breaking into parts outside the asci (HMAS-L 0137211); C, D. Asci, with eight biseriate ascospores (HMAS-L 0137211); E. Macroconidia (HMAS-L 0137209). ***Strigula laureriformis*** (KUN 7537). F. Thallus with pycnidia; G. Macroconidia. ***Strigula prasina*** (HMAS-L 0137213). H. Hypophyllous thallus; I. Asci, with irregularly arranged ascospores; J. Ascospores, fusiform-ellipsoid, 1-septate, with slight constriction at septum and not breaking into parts outside the asci. Scale bars: A = 200 µm; B–E = 10 µm, F = 800 µm, G = 20 µm, H = 300 µm, I, J = 10 µm.

Strigula prasina Müll. Arg., Flora 68: 343 (1885). Pl. 2H–J

Thallus hypophyllous, subcuticular, dispersed into patches, pale green.

Perithecia basally immersed, hemispherical, black. Paraphyses slightly branched. Asci dense and compact, cylindrical, 52.5–70 × 7.5–10 μm. Ascospores 8 per ascus, biseriate to irregularly arranged or occasionally uniseriate, fusiform-ellipsoid, 1-septate, with slight constriction at septum, 12–17 × 4–5 μm.

Pycnidia not seen.

Chemistry. No substances detected by TLC.

Specimens examined: **China**. **Hainan Province**, Ledong County, Jianfengling, on living leaves, 2009, J.C. Wei 046F1 (HMAS-L 0137213; GenBank KX216700), 046F2 (HMAS-L 0137212; GenBank KX216701).

Remarks: *Strigula prasina* is typically a hypophyllous species with a thallus morphology rather similar to that of *S. concreta*. However, *S. concreta* is typically epiphyllous and has distinctly smaller ascospores, often broken into parts (Santesson 1952). The other hypophyllous species, *S. janeirensis* (Müll. Arg.) Lücking, mainly differs in its thinner thallus, larger perithecia, and much larger ascospores that easily fragment within the asci (Roux & Sérusiaux 1995, Lücking 2008).

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